REMARKS

FORMAL MATTERS:

Claims 41-65 are pending after entry of the amendments set forth herein.

Claims 1-40 are canceled without prejudice.

Claim 52 has been amended to correct a clerical error.

New claims 54-65 have been added to more particularly point out and distinctly claim the invention. The newly added claims 54-59 are supported within Example 1 on pages 21-22 of the specification.

New claims 60-65 are also supported within Examples in the application and include language similar to that contained within claims 47-49.

No new matter is added.

RESTRICTION REQUIREMENT:

In response to the Restriction Requirement applicants canceled claims 1-40 and reserve the right to file a divisional application directed to the invention encompassed by any of these claims or any invention disclosed and described in the application.

CLAIM OBJECTION

Claim 52 is objected to due to a grammatical error. Applicants agree with the Examiner's position and the claim has been amended accordingly.

REJECTIONS UNDER 35 U.S.C. §102(E)

Claims 41-50 were rejected under 35 U.S.C. 102(e) as anticipated by U.S. Patent 6,602,711 to Thomson et al. The rejection is respectfully traversed for the reasons provided below.

Applicants claim a method of forming hESC aggregates wherein "the centrifugation causes aggregation of the hESCs." Further in new claims 54 applicants claim "centrifuging the suspension and thereby creating an aggregation of hESCs." Thomson et al. do not teach creating aggregation of hESCs by centrifugation.

At pages 3-4 of the Office Action, the rejection argues that claims 41-50 lack novelty over US 6,602,711 to Thomson. The argument focuses on the disclosure at column 4, lines 10-13, which describes centrifugation of an (embryonic stem) cell suspension as follows:

> After dispase treatment the colonies can either be washed off the culture dishes or will become free of the tissue culture plate with gentle agitation. After collagenase treatment the cells can be scraped off the culture dish with a 5 ml glass pipette. Some dissociation of the colonies occurs, but this is not sufficient to individualize the cells. **After chemical removal of the cells from the tissue culture plate, the cell suspension is centrifuged gently for 5 minutes, the supernatant is removed and discarded, the cells are rinsed, and the cells are resuspended in culture medium with or without serum.** (Bold added)

Thomson teaches a method for producing primate embryoid bodies from colonies of primate embryoid stem cells adhered to a substrate. The adhered colonies are removed in "clumps" *via* mechanical or chemical means and then incubated under non-attachment conditions (*i.e.* via continuous rocking/agitating of the culture dish and/or continuous culture in the presence of serum-free medium which lacks attachment factors) so as to facilitate the formation of embryoid bodies (column 2, line 40 to column 3, line 3; column 3, lines 24-35).

Thomson makes it clear that the adhered colonies are removed from the substrate *via* mechanical or chemical means so as to keep the embryonic stem cells in clumps (column 3, lines 63-65). The chemical release step involves the use of a chemical agent such as calcium disodium EDTA or a proteinase which acts on the extracellular matrix (*e.g.* dispase, collegenase, catalase, neuraminidase, pancreatin, pancreatin elastase or trypsin; column 2, lines 55-58); whereas mechanical removal is achieved using a pulled glass pipette to scrape the cells from the culture plate (column 4, lines 16-19). In Example 1, it discloses that after chemical removal of the cells from the tissue plate, the cell suspension (*i.e.* clump) is centrifuged for 5 minutes and resuspended in a culture media with or without serum (column 4, lines 5-15).

Thomson et al. use this particular approach for the production of primate embryoid bodies from colonies of primate embryonic stem cells for the reason explained at column 2, lines 23-43 of Thomson. It teaches that embryoid body formation using conventional murine protocols fails. In such conventional protocols, the embryonic stem cells are dispersed to single cells, and either allowed to

aggregate into embryoid bodies under conditions which prevent cell attachment to the substrate or allowed to grow into embryoid bodies from single cells or clusters suspended in methylcellulose. Thomson goes on to state that they have learned that primate embryonic stem cells die rapidly if dispersed to single cells and attachment is prevented.

Accordingly, Thomson teaches the importance of first aggregating cells on a substrate to form cell colonies, removing/isolating the aggregated colonies in cell clumps using mechanical or chemical means, and then incubating the cell clumps under non-attaching conditions in a (serum-free) culture media so as to produce embryoid bodies. In the context of the method taught by Thomson, the centrifugation step identified by the Examiner at column 4, lines 10-13, is simply used to isolate the cell clumps removed from the substrate by chemical means, prior to the incubation step. It is worth noting that after mechanical removal, no centrifugation step is involved (column 4, lines 18-19 of Thomson). Thus, Thomson et al. do not carry out the claimed step where centrifugation causes aggregation of the hESCs.

The present invention teaches a completely different method for producing embryoid bodies. In reference to Example 1 at pages 21-22 of the specification, human embryonic stem cells (hESCs) are grown on mouse feeder cells to 60-80% confluency, washed and resuspended in a differentiation medium (first without, and then with, growth factors). The cell suspension is then aliquoted into a low adhesion plate prior to centrifugation to form hESC aggregates. As identified by the Examiner at page 3 of the Office Action, the present specification at page 1, lines 28-34, makes it clear that embryoid bodies are defined as spheroids of cellular aggregates derived from one or a number of hESCs (in other words, the hESC aggregates referred to in Example 1 of the present application are in fact embryoid bodies).

Accordingly, both claims 41 and 54 are novel over Thomson because **the formation of embryoid bodies from a cell suspension** (where the cells are first dispersed to single cells) **is the direct result of a centrifugation step**. As discussed previously, the centrifugation step taught by Thomson is used to isolate the primate embryonic stem cell clumps prior to the incubation step, and it is only during the incubation step that the embryoid bodies are formed. Accordingly, **the centrifugation step disclosed in Thomson is not directly attributable to the formation of embryoid bodies**.

In addition, Thomson teaches the importance of first aggregating cells on a substrate to form cell colonies prior to the formation of embryoid bodies. However, this step is not taught in the methods of the present invention. In fact the present invention teaches the very opposite, because the resuspended

hESCs are aliquoted directly into each well of a 96 well round-bottomed untreated low adhesion plate (Nunc, cat # 264122) to facilitate aggregation (page 22, lines 12-14 of the present specification).

At pages 4-6 of the Office Action, the Examiner contends that claims 41 and 50-53 are obvious over Thomson in light of Kaufman *et al.* (2001) *Proc. Nat. Acad. Sci.* 98(19):10716-10721. The Examiner argues that while Thomson fails to teach the differentiation of blood cells from human embryonic stem cells, this is taught by Kaufman. The Examiner therefore asserts it would be obvious to combine the teaching of Thomson and Kaufman to arrive at a method for differentiating cells from hESC aggregates so as to form (*e.g.*) blood cells.

However, for the reasons indicated above Thomson et al. do not teach the claimed invention. Specifically, Thomson et al. do not teach a method wherein the centrifugation itself results in the creation of an aggregate of hESCs. In the absence of disclosing such the combination of Thomson et al. with Kaufman et al. does not teach the basic concepts of the invention. The basic method taught by Thomson et al. for producing primate embryoid bodies is quite different from the method claimed by applicants. Accordingly, one skilled in the art would not find it obvious to combine Thomson et al. with Kaufman et al. to obtain the claimed invention or arrive at the differentiation methods of claims 51-53. In view of such reconsideration and withdrawal of the rejections is respectfully requested.

CONCLUSION

Applicant submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number DVCC-009.

Respectfully submitted,
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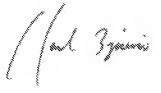

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